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FACTORS AFFECTING THE ANTHER CULTURE OF WHEAT (*Triticum aestivum* L.)

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ABSTRACT

The limitations of conventional cereal breeding programmes require simple and rapid new breeding technologies such as doubled-haploid production. One of the various techniques used to obtain in vitro haploids of wheat (Triticum aestivum L.) is anther culture. In this study the effects of cultivar, pre-treatment of spikes, callus induction media and carbon sources added into induction medium on anther culture response of Turkish wheats were investigated. Anthers from 5 commercial cultivars (Basri Bey, Golia, Marmara 86, Gönen and Pehlivan) grown in the field were excised and cultured on 2 nutrient media (N6 and Potato). Three cold pre-treatments (1, 2 and 3 weeks at +4 °C) and 2 carbon sources (sucrose and maltose) were tested. Hexaploid wheat cultivars showed low response to current anther culture technique. The 2-week-pre-treatment of spikes resulted in the highest callus induction whereas no callus was obtained from 3-week-pre-treatment. No significant differences was observed between the cultivars. Callus formation within cultivars varied between 3.0 and 7.0 per 1000 anthers plated. Basri Bey produced the highest callus formation among the cultivars used. Sub cultures of calluses on a regeneration media caused green zones. Callus induction medium containing sucrose caused a significant increase in callus production.

Introduction

Various haploid techniques may provide the breeders with large number of haploid plants by decreasing the time needed to improve a cultivar, compared to conventional methods (23). One of them is anther culture. Anther culture of wheat (*Triticum aestivum* L.), through which the production of homozygous progeny in one generation can be possible, is a highly desirable goal for researchers. There are many advantages of using anther culture. One of the most important advantages is to reduce the time of breeding cycles for double haploid production. This will allow the selection of desirable characters via *in vitro* methods in a short time and space. It is also used for the rapid selection of recessive alleles.

The first haploid plants from microspores of *Datura innoxia* Mill. were obtained from anther culture by Guha & Maheshwari (10). The confirmation of the process by Nitsch & Nitsch (19) with tobacco (*Nicotiana tabacum* L.) increased extensive interest in the culture of haploid plants. Although wheat androgenesis was first reported by three different groups in 1973 (2, 22, 24), there are still some problems in the use of haploids in plant breeding. The major problem is the limitation in haploid plant production from wheat anthers compared to other economically important plants such as rice, barley and rapeseed. Straub (28) noted that 1 anther produced 20 haploid plants in *N. tabacum* whereas 1 plant could be obtained from 10.000 wheat anthers.

However, in recent years many successful attempts to improve the anther culture methods of wheat have been achieved by many researchers (5, 9, 14, 25, 31). New hexaploid wheat cultivars such as 'Florin'

(4) and 'Delibab' (21) have been released by anther culture. On the other hand, in order to exploit the potential use of anther culture technique, it is necessary to develop the optimum techniques for the initiation and regeneration of culture. The response of anther culture is depend on many factors, including species (28), cold pre-treatment of spikes (16), developmental stage of microspores (13), genotype (5, 6, 8, 14, 20), growth environment of genotypes (7, 20), composition of culture media (31), culture conditions (15, 24) and the interactions among these factors (5, 7). The aim of this study was to develop an efficient and reliable haploid plant production procedure via anther culture applicable to Turkish hexaploid wheat genotypes.

Materials and Methods

The experiments were conducted at Uludağ University, Agricultural Faculty, Field Crops Department, Plant Tissue Culture Laboratory in 2000. Five wheat (*Triticum aestivum* L.) cultivars, Basri Bey, Golia, Marmara 86, Gönen and Pehlivan were evaluated in this study. Plants were grown in field. Spikes were collected when the top of the developing spike was level with the ligule of penultimate leaf. The anthers with microspores at mid-to late-uninucleate stage were used for inoculation (13). The pollen stage was also identified microscopically after staining the anthers in a drop of acetocarmine. The spikes were placed in flasks containing tap water and kept in a fridge in darkness at 3 to 5 °C. Spikes were left one week, two weeks and three weeks in the fridge for cold pretreatments. After that spikelets were cut 2/3 and sprayed with 70% (v/v) ethanol. They were immersed in 70% (v/v) ethanol for 10 sec, then surface sterilised with 3% NaOCl solution containing one drop of wetting agent for 20 min and rinsed six times in sterile distilled water. The anthers were cultured on N6 medium (1) and fresh potato-extract medium (3) for induction of callus cultures. Two carbon sources (sucrose and maltose) were added into each medium. Cultures were incubated at 28 ± 1 °C in the dark. At the end of 6 weeks, the number of anthers producing callus were counted. Anthers producing callus were transferred to a regeneration medium as defined by Schaeffer *et al.* (26). A total of 5571 anthers were cultured over a two month period.

Callus induction was calculated as the average number of callus obtained per 100 anthers plated. Data on callus induction obtained from individual petri dishes were considered as replication. Each replication consisted of one petri dish with 100 anthers. Statistical analysis were computed with Minitab programme. Data were analysed using the completely randomised design with 4 replications. LSD was used to determine the significant differences between treatments.

Results and Discussion

In total 30 anthers (0.54%) of the 5571 anthers cultured in this study responded with pollen callus. Mean callus induction frequency ranged from 0.33 to 0.69 and number of callus regenerated green zones from 1 to 5 (**Table 1**). When 3 cultivars treated with 3-cold pretreatments they did not produced significantly different amount of callus (**Table 3**). It is clear that cultivars produced almost the same amount of callus. This can be explained that these cultivars may originated from the same location of Turkey. Holme *et al.* (14) pointed out that the origin of the wheat was very important to reveal the anther culture response. Hexaploid wheat genotypes from north-western Europe revealed low responses to anther culture technique applied compared to eastern Europe. Extensive genetic variability was shown for all haploid regeneration parameters and yield traits of hexaploid wheat by Moieni & Sarrafi (18). Various scientists reported that genotype of the donor plants from which

TABLE 1

Anther culture responses of 5 wheat cultivars

CULTIVAR	Number of anthers cultured	Number of anthers responding	Frequency of anthers responding (%)	Number of callus regenerated green zones
Basri Bey	1263	7	0.55	4
Marmara-86	1186	7	0.59	3
Golia	1011	7	0.69	5
Pehlivan	1608	6	0.37	3
Gönen	503	3	0.33	1
TOPLAM	5571	30		16

TABLE 2

Analysis of variance and mean squares for callusing frequency of 3 wheat cultivars treated with 3 cold pre-treatments

Source	df	Mean squares
		Callusing frequency
Period	2	3.5278**
Cultivar	2	0.3611
Period x Cultivar	4	0.3611
Error	27	0.5093
Total	35	

**Significant effect at $\alpha = 0.01$

TABLE 3

Analysis of variance and mean squares for callusing frequency of 2 two-week-cold-pre-treated wheat cultivars grown in induction media with 2 carbon sources

Source	df	Mean squares
		Callusing frequency
Cultivar	1	0.063
Carbon source	1	5.063*
Cultivar x Carbon source	1	0.063
Error	12	0.81
Total	15	

*Significant effect at $\alpha = 0.05$

the anthers were taken was very effective on the haploid plant production (5, 14).

Despite the fact that, there was no significant differences between the cultivars in terms of callus production, cv. Basri Bey tented to produce slightly more callus structure than the other cultivars when 3 cold pretreatments were applied to anthers (**Table 2** and **Table 3**). Although all the cultivars tested responded well for production of callus from cultured anthers, they produced relatively low frequencies of callus per anther. The average frequency of anther producing callus is mainly similar to results of Hatipoglu *et al.* (11) but it is lower than the values obtained by De Buyser *et al.* (4), Ekiz & Konzak (8) and Hatipoğlu *et al.* (12)

The analysis of variance results of the first experiment is presented in Table 2. It shows significant differences between 3 cold pre-treatments for callus production frequencies at p=0.01 level. The 2-week-cold-pre-treatment of the spikes before inoculation was the most responsive. Similar result was observed by Hu & Zeng (15) when wheat anthers pre-treated at 1-4 °C. The frequency of callus induction increased about twice by 48 hrs. Xu *et al.* (30) also reported that cold pre-treatment of spikes and higher inoculation density of anthers resulted in a higher frequency of embryoid production in barley. With respect to callus production frequency significant differences exist within carbon sources used in the induction media. When sucrose used in the medium, the more callus induction was observed (**Tables 4** and **5**).

Callus induction was significantly influenced by neither cultivars nor induction media used. Although no significant differences was obtained between two different induction media used, the most suitable media for callus induction was N_6 with sucrose (data are not showed). Approximately N_6 and PM media induced the same number of callus per 100 anthers as indicated by Wei (29). N_6 medium has been considered a suitable medium for wheat anther culture for many researchers (17, 27). The contents of the major salts of potato tubers vary widely. Therefore, the effect of potato medium in callus induction medium was not always stable. The use of N_6

TABLE 4

Analysis of variance and mean squares for callusing frequency of 2 two-week-cold-pre-treated wheat cultivars grown in induction media with 2 carbon sources

Source	df	Mean squares
		Callusing frequency
Cultivar	1	0.063
Carbon source	1	5.063*
Cultivar x Carbon source	1	0.063
Error	12	0.81
Total	15	

*Significant effect at $\alpha = 0.05$

TABLE 5

Initiation of callus from anthers of 2 two-week-pre-treated wheat cultivars grown in induction media with 2 carbon sources

Cultivar	CARBON SOURCE		
	Sucrose	Maltose	Cultivar Means
Pehlivan	1.5	0.5	1.00
Marmara-86	1.5	0.25	0.88
Carbon Source Means	1.5 A	0.38 B	

Standard errors; Sx (Carbon source): 0.23; Sx (Cultivar): 0.23; Sx (Carbon source x Cultivar): 0.32

medium is more practicable than PM medium, since potato extract should prepare freshly whenever medium is prepared.

The number of embryoid, the frequency of callus or embryos regenerating plants should be examined in the future to determine the efficiency of the anther culture technique. Genotype specificity seemed to be a major obstacle in this study. The response of crosses made between the selected or new genotypes which has got different anther culture response can be determined in the future studies. It can be suggested that the genotypes with high frequency regeneration capacity should be used in order to successfully apply this technique into wheat breeding programmes. Moreover, it is also necessary to continue the optimisation of culture conditions in the future experiments. This technique is still too inefficient for a broad use of the method in wheat breeding. However, in this study promising results were obtained and this will encourage the use of anther culture technique with optimisation of the other factors.

From the results, it can be concluded that anther culture induction depended on the cultivar, growing conditions of donor plants and media composition.

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